# EXHIBIT 44

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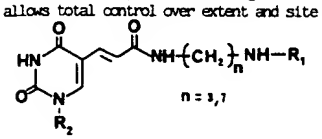
CHEMICAL SYNTHESIS OF MODIFIED OLIGONUCLEOTIDES AND THEIR UTILITY AS NON-RADIOACTIVE HYBRIDIZATION PROBES. Jerry L. Ruth* and Robert N. Bryan*, (SPON:N. KAPLAN), Molecular Biosystems, Inc., San Diego, CA 92121

Several deoxyuridine analogs with a primary amine "linker arm" of eight or twelve atoms in length attached at C-5 (**1**) have been efficiently prepared. These linker arm nucleosides ("LANs") are appropriately blocked and chemically incorporated into oligonucleotides in good yields. This method allows total control over extent and site of modification in the oligomer.




**1**: $R_1$ = H
$R_2$ = deoxyribose
**2**: $R_2$ = oligonucleotide
a) $R_1$ = H
b) $R_1$ = fluorescein
c) $R_1$ = caproylbiotin

Using a modified phosphochloridite method, three 22mers complementary to known sequences of herpes simplex virus (HSV) were constructed to be specific for either HSV-1, HSV-2, or both (generic). The purified oligomers were kinased and hybridized to HSV target DNA. Targets included an HSV-containing plasmid, lab strains of HSV, and clinical samples of both types 1 and 2. Results clearly demonstrated the desired selectivity. Oligonucleotides containing one or more LANs were selectively modified by attachment of fluorescein (**2**b) or biotin (**2**c). Biotinylated probes (**2**c) kinased and hybridized normally. Non-radioactive detection of **2**c was accomplished by dye formation using an avidin-dehydrogenase complex. Results indicate such modified oligonucleotides can: 1) be made very efficiently by chemical methods in large amounts; 2) detect target DNA cleanly and specifically; and 3) be detected by non-radioactive methods.

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SYNTHESIS, THERMODYNAMICS AND DRUG INTERACTIONS OF MODEL OCTANUCLEOTIDES. I. Lassalle*, H.L. Weith and S.R. Byrn*. Purdue University, West Lafayette, Indiana 47907.

Two pairs of complementary octanucleotides were chemically synthesized using the modified phosphotriester method. Their sequences are: TGACGTGA - TCACGTCA and TGAGGTGA - TCACCTCA. Optical studies were performed on the single strands in order to accurately determine their molar absorptivity coefficient (ε) and their % hyperchromicity. The thermodynamics of helix formation were determined from melting curve data. In addition, the binding of the acridine AMSA to both the single strands as well as the duplexes was investigated. This data provides insight into the parameters controlling helix formation and drug binding.

Sponsored in part by grants CA30234 and GM29175.



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LAMBDA PHAGE Cro REPRESSOR-DNA INTERACTIONS: FLUOROURACIL ANALOGUES OF $O_R3$. William T. Metzler*, Elizabeth Tecza*, Kim T. Arndt* and Ponzy Lu. Department of Chemistry, Univ. of Pennsylvania, Philadelphia, PA 19104

We have synthesized 15 base pair analogues of the center of the lambda $O_R3$ DNA sequence which contain deoxyfluorouracil replacements at specific thymine locations, 5 and 10, in the sequence below [indicated by * using the numbering system of Kawashima et al. (1977), Biochemistry 16, 4209-4216]:

```
  2  5    10   15
  .  .    .    .
ATCACCGCAAGGGAT
TAGTGGCGTTCCCTA
   *    *
```

Using quenching of the fluoresence at the tyrosines of the cro repressor molecule, we were able to demonstrate that the fluoroanalogue of $O_R3$ binds to the protein as well as a 15 base pair non fluorine containing $O_R3$ sequence.

By observing the Fluorine-19 NMR signals in the presence and absence of the cro repressor we are able to monitor the formation of the specific cro-$O_R3$ complex. (Supported by grants from the NIH.)

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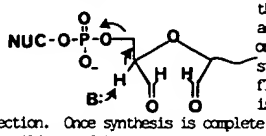
A NOVEL UNIVERSAL SUPPORT FOR DNA & RNA SYNTHESIS. L. J. Arnold, Jr.*, R. Lohrmann*, and J. L. Ruth*(SPON: N. O. Kaplan), Molecular Biosystems, Inc., San Diego, CA 92121

Current methods of DNA and RNA synthesis have been greatly facilitated by the use of solid supports. Typically such supports have either a silica or organic matrix to which is attached the first nucleoside through a 3' ester linkage. Since the nucleoside can be any of four ribo- or deoxyribonucleosides, eight such supports are necessary to carry out RNA and DNA synthesis. We have designed a universal support which uses a ribose as a primer with




the 2' and 3' OH protected by substituted acetyl groups. The support matrix is a organic graft which is non-swellable, is stable to acid and base, and has superior flow properties. DNA or RNA synthesis is carried out in either the 3' or 5' direction. Once synthesis is complete, the ribose acetyl groups are removed with mild base followed by the selective oxidation of the cis diol by periodate and a β-elimination of the formed oligomer. This support has many advantages which include: 1) It is equally useful for RNA or DNA synthesis; 2) Chain elongation may go in either the 3' or 5' direction; 3) The synthesized oligomer may be base and phosphate deblocked before or after removal of the oligomer from the support; 4) The product oligomers are of higher purity since blocking groups can be removed before removal of the oligomer; and 5) After removal of protecting groups the oligomer may be left attached which permits its use as an affinity hybridization support. The functional properties of this support have been confirmed using $^{14}$C-thymidine labels. Under deblocking conditions, less than 6% of the oligomer is removed. During selective cleavage greater than 80% of the oligomer is eliminated. Using this support we have synthesized a range of 14-20 mers.

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NEW METHOD FOR SEQUENCE ANALYSIS OF OLIGODEOXYRIBONUCLEOTIDES. D. M. Black* and P. T. Gilham. Purdue Univ., West Lafayette, IN 47907.

A simple sequencing procedure employs a two-dimensional separation on a single thin layer chromatographic sheet and consists of, in the first dimension, the fractionation by chain length of a nested set of fragments derived from the oligodeoxyribonucleotide by partial exonuclease action and labelled at their non-common ends, followed by in situ enzymatic degradation of the members of the set and separation of the resulting labelled mononucleotides in the second dimension. Spleen phosphodiesterase and alkaline phosphatase are used to produce the nested set whose members have a common 3' terminus, and these are radioactively labelled at their non-common ends with polynucleotide kinase. The fragments are separated on a polyethyleneimine-cellulose thin layer sheet, using a modification of the solvent system developed by Randerath et al. [Nucleic Acids Res. 1, 1121 (1974)]. The oligomers are then digested in situ to 5' mononucleotides using nuclease Bal 31, nuclease S1, or snake venom phosphodiesterase, and the labelled monomer formed at each position is identified by chromatography in the second dimension with 0.2 M NaOAc adjusted to pH 4.2 with AcOH. This approach to sequence analysis has an added advantage in permitting assignment of the identity and location of any modified or unusual base within the oligonucleotide.
(Supported by CA 30234)

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NON-RADIOACTIVE BIOTIN - DEPENDENT HYBRIDIZATION/DETECTION USING UNLABELED PROBE DNA. BY: CHRISTINE L. BRAKEL*, KATHERINE MARKARIAN, AND DEAN L. ENGLEHARDT. ENZO BIOCHEM, INC. NEW YORK, NEW YORK 10013.

We have developed a method for the non-radioactive detection of DNA probes that do not contain modified nucleotides. In this system, the probe DNA is 3' terminally labeled with TTP or dATP in the presence of terminal transferase to result in a probe which contains single stranded homopolymeric terminii. After this probe has been hybridized to target DNA, the hybridized molecules are complexed with a complementary homopolymer containing biotinylated nucleotides. Hybridization is visualized using a colormetric detection system for biotin composed of streptavidin (a biotin-binding protein) and a biotinylated enzyme (either acid phosphatase or horseradish peroxidase). Addition of the appropriate substrate yields a colored precipitate. The detection sensitivity of this system was found to be greater than the sensitivity obtained using directly biotinylated probes, prepared either by nick translation or terminal labeling. This new method can be used for the detection of specific sequences on dot blots and Southern transfers.

# American Society of Biological Chemists

75th Annual Meeting

*and*

# The American Association of Immunologists

68th Annual Meeting

St. Louis, Missouri

June 3–7, 1984

## ABSTRACTS OF PAPERS

2477–3780

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VOLUME 43, NUMBER 7 · MAY 4,

# Federation Proceedings

Official Publication of th
FEDERATION OF AMERICAN SOCIETIE
FOR EXPERIMENTAL BIOLOG

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It is our pleasure to record the help of the staff of BIOSIS in preparing the indexes, particularly from Ms. Carolyn Marconi and Mr. John R. Thomas.